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Isolation and Identification of Aerobic Bacterial Flora in Nasopharyngeal Passageways of Apparently Healthy and Clinically Sick Sheep at Gondar University Veterinary Clinic

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Abstract: A cross sectional study was conducted from November, 2010 to April, 2011 with the aim of isolating and identifying aerobic bacterial flora in nasopharyngeal passageways of apparently healthy and clinically sick sheep at Gondar University Veterinary Clinic. Samples were collected aseptically from the nasal cavity of 142 investigated sheep and standard microbiological techniques were used for isolation and identification of bacterial species. From the total of 142 specimens (74 healthy and 68 clinically sick sheep) collected for bacteriological examination, 136 contained bacteria. A total of 152 bacteria were recovered in which 77 and 75 of them were from apparently healthy and clinically sick, respectively. The isolation rates of frequently identified isolates recovered from healthy sheep include; *S. epidermidis* (15.6%), *E. coli* (14.3%), *C. pseudotuberculosis* (11.7%) and *S. aureus* and *M. luteus* (9.1% each),. On the other hand, isolation rates of most frequently identified species among the 75 bacterial isolates include; *S. aureus* (17.3%), *S. epidermidis* (13.3%), and *M. haemolytica* and *M.luteus* (10.7% each).The majority of the isolates which colonize the nasal cavity of apparently healthy sheep were also recovered from clinically sick sheep with the exception of *A. pyogenes* and *P. aeruginosa.* In conclusion, most of the isolated bacteria commonly act as primary pathogens; however, *Pasteurella and Mannhaemia spp,* are recognized as opportunistic pathogens capable of causing disease if they pass the normal defenses of the host.

Key words: Aerobic % Bacteria % Nasopharyngeal % Sheep % Nasal Swaps % Gondar

INTRODUCTION

Respiratory diseases of various etiologies have been described in different domestic animals. However, the problem is more common in sheep due to the fact that the ratio of the alveolar surface to metabolic weight is very low in sheep compared to other species [1]. Many specific primary pathogens have been implicated sheep pneumonia. The most common bacterial causes of pneumonia in sheep include *pasteurella spp., Mannheimia haemolytica, Actinomyces pyogenes* and several *mycoplasma species*. *P. multocida* and *m.haemolytica* are important contributory pathogens in enzootic or primary pneumonia in sheep, although their pathogenic effects are enhanced when sheep are infected with viruses [2].

Although a single agent may be the primary invader, most respiratory infections are complicated by the presence of secondary or opportunistic invaders [3]. When the local resistance of respiratory mucus is lowered, bacteria growing in the nose and throat extend down wards, usually producing multiple bacterial infections [4]. Besides, most of the infectious agents that cause respiratory disease are ubiquitous in nature and are normal inhabitants of the nasopharynx of normal animals. This often creates difficulty with the interpretation of microbiological findings in outbreaks of respiratory diseases [5].

There are several studies conducted on both healthy and pathological respiratory tracts of cattle, camel and small ruminants in different regions of the country [6, 12]. However, their works, particularly in sheep, were

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restricted to the study of bacterial flora of pneumonic lungs and less emphasis was given to the bacterial flora of apparently healthy respiratory passageways of sheep. Therefore, the objectives of this study were to determine the type of normal bacterial inhabitants in nasopharyngeal passageways of apparently healthy and clinically sick sheep, establish the extent of normal bacterial inhabitants of nasal cavity that serves as a benchmark in the diagnosis and treatment of respiratory disease out breaks and identify potential bacterial species that may be considered for future research work in etiological identification and development of vaccine.

MATERIALS AND METHODS

The study was conducted from November 2010 to April 2011, in Gondar town particularly at Gondar University Veterinary clinic The production system observed around the area combines cereal-based agriculture and livestock farming.

Study Animals: The study animals were 142 local highland sheep (both males and females), which were brought to Gondar University Veterinary clinic from different areas of Gondar town and its surroundings. The animals were raised in traditional management practices. Records concerning the exact age and pervious history of management or health status of sheep were not available; however, some of them (74) were found to be clinically normal while others (68) were clinically sick during clinical examination.

Study Design: A cross sectional study was carried out from November 2010 through April 2011 to isolate and identify bacterial flora from nasal swab specimens taken from the nasal cavity of sheep (females and males) each week. These animals were comprised clinically as normal and clinically sick at Gondar University Veterinary clinic.

Sample Collection: Nasal swabs were collected in sterile test tubes after cleaning and disinfecting the external part of the nose using 70% alcohol. The swabs were replaced back into the tubes to which a transport medium (3 ml of tryptone soy broth) was added. The test tube containing the swab was labeled and kept in an icebox and transported to the Gondar University Veterinary Microbiology Laboratory for further processing. In the laboratory, samples were immediately incubated aerobically at 37°C for 24 hours [13].

Bacteriological Examination: The cultured broth samples were thoroughly agitated and mixed. A Loop full of the cultured broth was streaked onto identified blood agar plate supplemented with 7% sheep blood.

Isolation and Identification: The growth of typical colonies was characterized concerning the presence and type of hemolysis (Blood agar), ability to ferment lactose (Mac Conkey agar) and general appearance of colonies on both media. Pure cultures of a single colony type from blood and MacConkey agar were transferred to nutrients agar media for a series of primary and secondary biochemical tests [14].

Data Collection and Analysis: Infection (colonization) rates were recorded for each study animals. Descriptive statistics were used to summarize the data generated from the study. Finally relative abundance of each bacterial species was expressed as percentage in comparison to the total number of isolates and in relation to the animals' sampled (apparently healthy and clinically sick sheep).

RESULTS

From a total of 142 swab samples collected from the nasal passage of 74 apparently healthy and 68 clinically sick sheep, 136 of them contained bacteria. In general, a total of 152 bacteria was isolated from 136 infected specimens, (1.12 bacteria per infected sample). Out of the 152 isolated bacteria, 77 of them were recovered from apparently healthy (74) in which 54 (70.1%) and 23 (29.9%) were G^{+ve} and G^{-ve} bacteria, respectively and the rest 75 were recovered from clinically sick sheep (62) in which 53 (70.7%) and 22 (29.3%) were G^{+ve} and G^{-ve} bacteria, respectively (Table 1). Thirteen different bacterial species were isolated from the nasal cavity of apparently healthy animals sampled whereas from clinically sick sheep 11 species were recovered (Table 1).

The most frequently isolated species from apparently healthy animals were *Staphylococcus epidermidis* (15.6%), *Escherichia coli* (14.3%), Corynebacterium *pseudotuberculosis* (11.7%) *Staphylococcus aureus* and *Micrococcus luteus* (9.1% each). On the other hand, *Pseudomonas aeruginosa* (2.6%) and *Pasteurella multocida* (1.3%) were the least encountered bacterial species among the isolates (Table 1). The predominant species among the 10 isolates recovered from the nasal cavity of clinically sick sheep were *S. aureus* (17.3%), *S. epidermidis* (13.3%), *M. haemolytica* and *M. luteus* (10.7% each*). P. multocida* (2.7%) was the least encountered among the isolates (Table 1).

Table 1: Isolation rate of aerobic bacterial species form nasal cavity of apparently healthy and clinically sick sheep.

Bacterial spp.	Healthy (n=74)	Sick (n=68)
G+ve Bacterial spp.	Frequency (%)	Frequency (%)
A. pyogenes	4(5.2%)	0(0)
B. coagulans	5(6.5%)	7(9.3%)
C. psudotuberculosis	9(11.7%)	7(9.3%)
M. luteus	7(9.1%)	8(10.7%)
S. aureus	7(9.1%)	13(17.3%)
S. epidermidis	12(15.6%)	10(13.3%)
S. saprophyticus	6(7.8%)	4(5.3%)
S. Zooepidermicus	4(5.2%)	4(5.3%)
Subtotal	54 (70.1%)	53 (70.7%)
G-ve Bacterial spp		
E. coli	11(14.3%)	5(6.7%)
C. diversus	5(6.5%)	7(9.3%)
M. haemolytica	4(5.2%)	8(10.7%)
P. maltocida	1(1.3%)	2(2.7%)
P. aeruginosa	2(2.6%)	0(0)
Subtotal	23 (29.9%)	22 (29.3%)
Total	77(100%)	75(100%)

The majority of isolates colonize the nasal cavity of the examined animals with the exception of *A. pyogenes* and *P .aeruginosa* which were absent from the nasal samples of clinically sick sheep as shown in Table 1.

Gram positive bacteria were dominant over Gram negative in both apparently healthy (70.1% Vs 29.9%) and clinically sick sheep (70.7% Vs 29.3%) in this environment (Table 1).

Table 2 was deleted as there wasn't any comment about it in the abstract, results and discussion. Also the title of the table was concerned in determining the proportion of isolated bacterial species in relation to sampled animals but the calculated figures were in relation with the No. of isolated bacteria (152) while Table 1 was concerned with that.

DISCUSSION

The study has showed a wide variety of bacterial species inhibited and colonized the nasal passage of apparently healthy and clinically sick sheep. Several workers isolated similar bacteria from pneumonic caprine lungs [15-18]; apparently healthy respiratory tract and nasal cavity of goats [12, 19- 21] and with fewer reports from apparently healthy sheep [22].

The invariable isolation of these organisms from the nasal cavity of apparently healthy and clinically sick sheep in this study reflects their possible role in respiratory syndrome. The normal bacteria of healthy individual animal can be altered by several factors such as the nutritional and immunological status of the animal or the environment. The suppression of the normal bacteria frequently allows the development of potential pathogens, leading to the presentation of a variety of pathologies [21].

The pathogenic bacteria isolate, *Mannheimia haemolytica* was isolated in higher proportion from the nasal passage of clinically sick (10.7%) than apparently healthy sheep (5.2%) (Table 1). *M. haemolytica* was isolated in the nasopharynx tonsils of apparently healthy animals, where interestingly, serotype A2 is most commonly isolated from both sheep and cattle [19, 23-24]. Other previous workers [25] reported high incidence rate (55.46%) from pneumonic lungs. *Mannhaemia haemolytica* which is normal flora of upper respiratory tract may play a secondary role after the primary initiating agent suppress the host defense mechanism and favors the multiplication of Pasteurella species leading to bronchopneumonia [26].

In the current study, *Pasteurella multocida* was recovered in low proportion from the nasal passage of clinically sick and apparently healthy sheep (Table 2). *Pasteurella multocida* frequently inhabited tonsil and nasal cavity. This is consistent with previous reports from nasal cavity of apparently healthy caprine [19], but much higher rate has been reported from nasal cavity (34%) of apparently healthy caprine [12], indicating that the organism lives as a commensal in the upper respiratory tract, invading the lung under conditions of stress.

In agreement with previous reports [8, 22, 27, 28], both in terms of infection intensity and pathogenicity, it seems that *M. haemolytica* assumes greater prominence than Pasteurella in this environment.

The finding that *Staphylococcus epidermidis* was the more predominant species in healthy sheep (15.6%) compared to clinically sick (13.3%) indicating that this organism normally inhabits the upper respiratory tract. This finding conforms well to the previous study similarly isolated relative proportion rate (12.5%) of CNS from the nasal cavity of apparently healthy sheep [22]. *Staphylococcus saprophyticus* was the second CNS recovered from nasal passage of healthy (7.8%) and clinical sick animals (5.3%). The relatively low proportion of this isolate might indicate that clinically presented sheep were not suffered from pharyngeal and lung abscesses. CNS often involves in the pharyngeal and lung abscesses and suppuration of the remaining respiratory tract when the defense mechanism of the host is compromised [28, 29]. Shemsedin [11] and Shigidi [30] previously isolated, CNS from pneumonic camels lung.

Staphylococcus aureus was reported in higher proportion from clinically sick (19.1%) than from healthy nasal passages (9.5%) (Table 1). Although this finding is consistent with reports from the nasal cavity of apparently healthy caprine [12] and ovine lung [18, 25] much higher rates have been reported from pneumonic caprine lungs [25, 31]. Conversely, lower *Staphylococcus aureus* isolation rate (6.25%) was recovered from nasal cavity of healthy sheep [22]. According to Robbins *et al*. [28], *S. aureus* is the main inhabitant of the mucus membranes of the upper respiratory tract and opportunistically involves them in pathologic role following stress conditions, such as infection by influenza virus and can be become a serious cause of infection in immunosuppressed hosts.

E. coli was the second dominant bacteria inhabiting the nasal passage of apparently healthy sheep (14.3%); although isolation rate from clinical sick was lower (Table 1). This finding is in agreement with previous study involving apparently healthy ovine nasal cavity [22], apparently healthy caprine nasal cavity [12], normal rabbits [32] and condemned goats' lung [33]. Although *E. coli* is considered to be transient in the respiratory tract when inhaled with dust particles and do not play a pathogenic role [19], its isolation from clinically healthy sheep in the absence of clinical enteritis is noteworthy. Megra *et al*. [12] and Emikpe *et al*. [19] Suggested that *E .coli*, which is usually harmless in their normal habitat, could cause pulmonary and urogenital tract infections.

Corynebacterium pseudotuberculosis was isolated from 11.7 and 9.3% of the nasal samples of apparently healthy and clinically sick animals, respectively. Previous workers [12] isolated the organism from nasal cavity and tonsils of apparently healthy goats, while others [11, 30] isolated it from the nasal cavity and tonsil of camels. In line with this, Yimer and Asseged [22] reported from the presence of *C. pseudotuberculosis* in the nasal cavity, tonsils, trachea and lung with relative abundance in the tonsils and lung. It has been established that *C. pseudotuberculosis* is inhaled in to the respiratory tract from skin and mucous membranes [34].

Although micrococcus species were isolated from nasal cavities of normal rabbit [32], camels [11] and relatively higher prevalence of this species was found in the nasal cavity of clinically sick (9.1%) and healthy (10.7%) sheep in this study, they are assumed to be non-pathogenic [34]. Their ubiquity compared to other non-pathogenic species is primarily due to contamination from skin.

Other non pathogenic organism, *Bacillus coagulans* was recovered mainly from nasal samples of clinically sick (9.3%) than apparently healthy (6.5%) sheep. Similarly, Megra *et al*. [12] reported the isolation of *bacillus spp.* mainly from the nasal cavity (22%) followed by tonsils (10%) of healthy caprine. On the other hand, Ajuwape and Aregbesola [32] could not isolate the organism from the nasal swabs of normal rabbits, indicating that the organism preferentially is involved in diseased organs.

About 5.3 and 5.2% of the nasal samples taken from clinically sick and healthy sheep, respectively, contained *Streptococcus zooepidermicus*. Similarly, Yimer and Asseged [22] isolated *S. zooepidermicus* from nasal cavity of apparently healthy sheep, with highest incidence in the tonsil (12%). Azizollah *et al*. [21] indicated that *streptococcus spp.* is widely distributed in nature and lives as commensal in the respiratory tract of many species of domestic animals. Potentially pathogenic and non-pathogenic species might be present in the upper respiratory tract [34].

Actinomyces pyogenes was isolated from the nasal cavity of apparently healthy sheep (5.2%) where as clinically sick samples were free (Table 1). It has been stated that *A. pyogenes* is the commensal of the nasopharyngeal mucosa and causes disseminated purulent infections when the animal is stressed [29]. It may also reside as a commensal on the respiratory linings with a threat of causing pulmonary abscess and bronchopneumonia [22].

Citrobacter diversus frequently inhibited the nasal cavity of 9.3% clinically sick sheep compared to 6.5% healthy ones. Similarly, *Citrobacte*r s*pp*. was isolated from the nasal cavity of healthy sheep [22].

Pseudomonas aeruginosa was only reported from the healthy nasal samples where as from clinically sick sheep was not recovered (Table 1). Ajuwape and Aregbesola [32] similarly isolated *P. aeruginosa* from the nasal cavity of normal rabbits and pneumonic lung of sheep [33]. Yimer and Asseged [22] reported that *P. aeruginosa* is more prevalent in the trachea. *Pseudomonas aeruginosa* can be found on the skin, mucus membranes and in the feces of sheep and goats [13].

This study has indicated that a wide variety of bacterial species inhabit the nasal passages of apparently healthy and clinically sick sheep. In addition the present study provided a key role regarding bacteria commonly encountered in the upper respiratory passage ways of healthy animals. Most of these bacteria isolated from

the healthy and clinically sick sheep act as primary pathogens; however, *Pasteurella* and *Mannhaemia* spp. are recognized as opportunistic pathogens capable of causing disease if they pass the normal defenses of the host.

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